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Production of (+)-5-deoxystrigol by *Lotus japonicus* root culture

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Abstract

Lotus japonicus roots, cultured in a modified B5 medium, produced and secreted germination stimulants that induced *Striga hermonthica* seed germination. The germination-inducing activity was detected both in the roots and the culture filtrate. Following bioassay-guided purification procedures, an active compound was isolated from hexane extracts of the roots and the culture filtrate. Based on chromatographic behaviour on HPLC, and ^{1}H NMR, UV, MS and CD spectroscopic analyses, the germination stimulant was identified as (+)-5-deoxystrigol.

Keywords: *Lotus japonicus*; Leguminosae; *Striga hermonthica*; Orobanchaceae; Root culture; Germination stimulant; (+)-5-Deoxystrigol

1. Introduction

Striga and *Orobanche* species (Orobanchaceae) are obligate root parasitic weeds, which survive only when attached to the roots of appropriate host plants. They deprive water and nutrients of the host plants and cause devastating effects on their hosts. *Orobanche* is a holoparasite, which lacks chlorophyll, while *Striga* is a hemiparasite and can fix some but not all of its own carbon. Seed germination requirements of these parasitic weeds include an after-ripening period of several months, followed by preconditioning in a warm and moist environment for one to two weeks, and finally exposure to specific chemical signals, including strigolactone compounds (Butler, 1995). Classically the strigolactones have been described as sesquiterpene lactones; however, little was known about their biogenesis until Matusova et al. (2005) reported detailed experiments using *vp*14 maize mutants. They proposed that strigolactones are a product of carotenoid cleavage and (+)-5-deoxystrigol (**1**) is a key intermediate for the biosynthesis of a series of strigolactones. Isolation of (+)-5-deoxystrigol (**1**) from hydroponic culture of *Lotus japonicus* as a branching factor for arbuscular mycorrhizal fungi (Akiyama et al., 2005), lent credit to the proposed biogenesis and revealed an ecological significance of strigolactones (Hamphrey et al., 2006).

Plant tissue culture with high productivity of a target molecule is useful for investigating the biosynthetic pathway of the metabolite because the culture is kept aseptic and cultural parameters are easily manipulated. We have previously reported production of (+)-strigol (**2**) by *Menispermum dauricum* root culture (Yasuda et al., 2003). Isolation of the strigolactone from the aseptic root culture provided the first unambiguous demonstration that strigolactones are genuine products of the plant roots. The isolation also suggested that strigolactones occur even more widely in the plant kingdom. In the present study, we report the isolation of (+)-5-deoxystrigol (**1**) from *L. japonicus* root culture, the second example of *in vitro* production of a strigolactone.

2. Results and discussion

Root tips were collected from aseptic *L. japonicus* seedlings and cultured on a rotary shaker in the dark in liquid B5 media (Gamborg et al., 1968) containing various

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concentrations of indole-3-butyric acid (IBA), indole-3-acetic acid or 1-naphthaleneacetic acid. Preliminary experiments showed that the media containing 5 μM IBA gave the highest root growth and *Striga hermonthica* seed germination-inducing activity. The culture media, in which no roots were grown, did not stimulate *Striga* seed germination. Temporal changes of root growth, activity in the culture filtrate, and hexane and EtOAc extracts sequentially prepared from the culture filtrate were examined (Fig. 1). Roots grew well, with about a 60-fold increase in 20 days. Formation of the active compounds accelerated as roots entered late-log phase, typical to the formation of secondary metabolites (Sugimoto et al., 2007). Finding the activity in the hexane extracts suggested that the roots produce and secrete less polar stimulant(s) than (+)-strigol (**2**), which was not extracted by an apolar solvent (Yasuda et al., 2003). In the light of a previous finding that production of strigolactones by red clover roots was stimulated under low phosphate conditions (Yoneyama et al., 2001), *L. japonicus* roots were cultured in media with different concentrations of phosphate for 20 days and germination-inducing activity in each of the culture filtrates was evaluated (Fig. 2). At 10 and 100 μM, the activity was much higher than that at 1000 μM, the original phosphate concentration in B5 medium (Gamborg et al., 1968). No significant difference in the activity was observed between 10 and 100 μM, but since the latter gave a better root growth than the former, the medium with 100 μM phosphate was employed for further work. Phosphate concentrations did not affect pH values in the media after 20 days of culture.

L. japonicus roots were cultured for 20 days, and then roots and culture filtrate were collected separately. Based on preliminary experiments in which hexane extracts obtained from the roots and the culture filtrate gave the active compound with the same chromatographic behaviour at a final step of purification by HPLC, both of the hexane extracts were combined and subjected to purification procedures. The combined extracts were separated

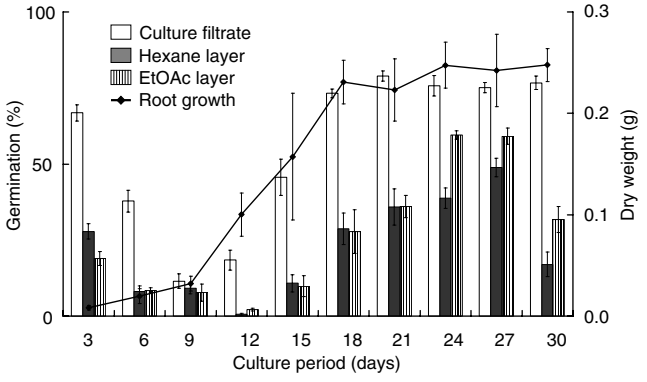

Fig. 1. Time course of *L. japonicus* root growth and germination-inducing activity in the culture filtrate. Excised roots (ca. 3.5 mg dry wt.) were cultured in 200-ml flasks containing 50 ml of medium. Culture filtrate was treated first with the same volume of hexane, and then with EtOAc. Data are the means ± SE ($n = 3$).

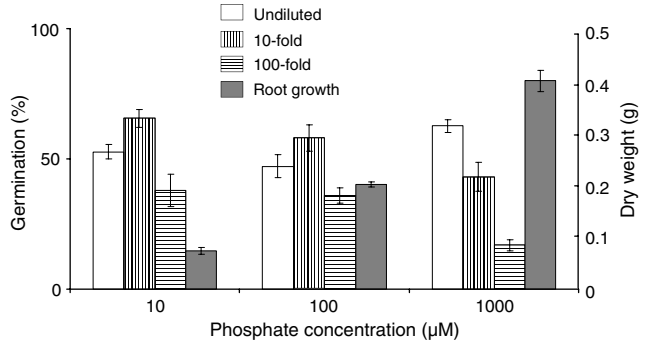

Fig. 2. Effects of phosphate concentration in culture media on *L. japonicus* root growth and germination-inducing activity in the culture filtrate. Data are the means ± SE ($n = 5$).

by Sephadex LH-20 column chromatography using a mixed solvent of MeOH–$CHCl_3$ (4:1) (Fig. 3a). Residues obtained from active fractions 14–22 were subjected to further Sephadex LH-20 column chromatography using MeOH–H_2O (1:1) (Fig. 3b). The active fractions 21–42 in the second chromatography were combined, MeOH was removed, and then active components were extracted with EtOAc from the aqueous phase. After removing the EtOAc, the residue was further purified by semi-preparative HPLC, resulting in the separation of a highly active

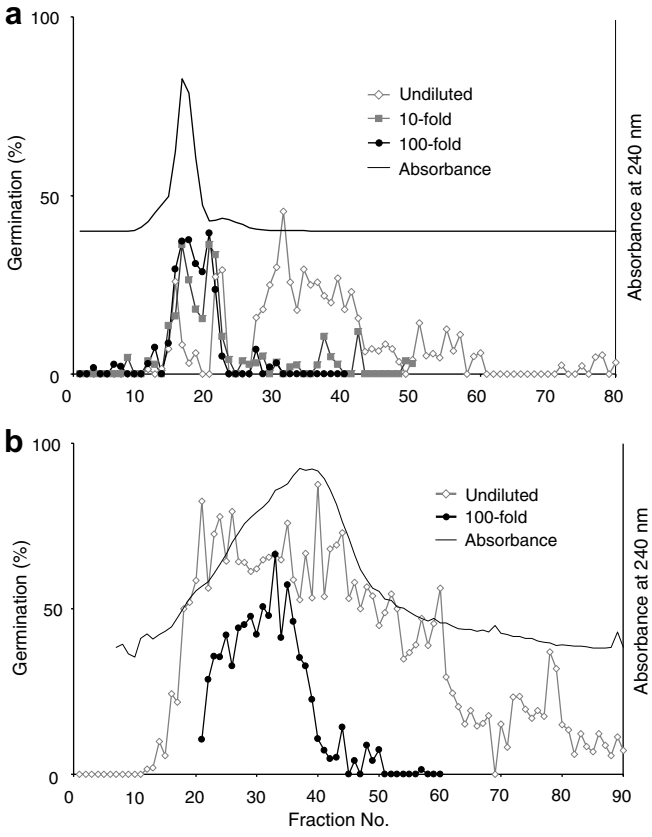

Fig. 3. Separation of the germination stimulant extracted from *L. japonicus* roots and culture filtrate by LH-20 chromatography. MeOH–$CHCl_3$ (4:1) (a) and MeOH–H_2O (1:1) (b) were used as a mobile phase.